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(54) PLATELET DERIVED GROWTH FACTOR EXPRESSION SUPPRESSOR

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a platelet derived growth factor expression suppressor, useful as a therapeutic agent for interstitial pneumonia, comprising a specific anti-sense oligonucleotide as an active ingredient.
SOLUTION: This suppressor comprises an anti-sense oligonucleotide to an arbitrary domain of a B chain of a platelet derived growth factor (PDGF for short) as an active ingredient. The typical sequence of the anti-sense oligonucleotide as the active ingredient includes, for example, a PDGF-B chain exon 4 domain, exon 1 domain, a splicing site-containing domain, a domain between exon 2 and exon 3, an exon 5 domain, a domain between exon 4 and exon 5, an exon 6 domain or an exon 7 domain. An anti-sense oligonucleotide selected from sequence numbers 1-18 may be cited as the anti-sense oligonucleotide.

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CLAIMS

[Claim(s)]
[Claim 1] The platelet derived growth factor manifestation inhibitor which makes an active principle the antisense oligonucleotide to the field of the arbitration of the B chain of a platelet derived growth factor.
[Claim 2] The platelet derived growth factor manifestation inhibitor according to claim 1 whose field of arbitration is a field of an exon 4.
[Claim 3] The platelet derived growth factor manifestation inhibitor according to claim 1 whose field of arbitration is a field of an exon 1.
[Claim 4] The platelet derived growth factor manifestation inhibitor according to claim 1 which is the field where the field of arbitration includes a splicing site.
[Claim 5] The platelet derived growth factor manifestation inhibitor according to claim 1 whose field of arbitration is the field between an exon 2 and an exon 3, the field of an exon 5, the field between an exon 4 and an exon 5, the field of an exon 6, or a field of an exon 7.
[Claim 6] The platelet derived growth factor manifestation inhibitor according to claim 1 which is what is chosen from that an antisense oligonucleotide is indicated to be by the array numbers 1-18.
[Claim 7] The platelet derived growth factor manifestation inhibitor according to claim 1 to 6 which is a pneumonitis therapy agent.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the manifestation inhibitor of a platelet derived growth factor (Platelet derived growth factor, PDGF), and a more detailed PDGF manifestation inhibitor useful as a pneumonitis therapy agent.
[0002]
[Description of the Prior Art] The intervention of various kinds of cytokine is submitted to the fibrosis of the lungs in pneumonitis. That is, the fibrosis of lungs is understood to be a trauma recovery process, and the fibroblast which constitutes a mesenchyme system cell, a smooth muscle cell, the macrophage of the corpuscle cell origin, a lymphocyte, neutrophil leucocyte, eosinophile leucocyte, basophilic leucocyte, the alveolar cell as an epithelial cell, the respiratory-epithelium cell, the vascular endothelial cell, etc. are known as a cell population which participates in this. Respectively it activates, and these cells cause different gene expression from the bottom of health condition, and discover various cytokine etc., and the change manifestation of adhesion molecules also makes them cause it in inflammation stimulus Shimo etc.
[0003] As cytokine in which a production manifestation is carried out by these various cells For example, although interleukin (IL-1alpha, IL-1beta, IL-6, IL-8 grade), CSF (GM-CSF, G-CSF, M-CSF, etc.), TNF(s), TGF(s), IFN(s), growth factors (EGF, TGF, PDGF, IGF, etc.), etc. are known Playing the role important for the fibrosis lesion of lungs among these it is reported that IL-6, IL-8, TGF-beta, etc. are PDGF(s) -- [-- for example, the 34th Japan chest disease and the collection of lecture summaries -- 191 pages, 192 pages (the 1994 fiscal year), the 35th Japan chest disease, and the collection of lecture summaries -- reference], such as the 232nd page (the 1996 fiscal year).
[0004] Above PDGF was found out by the Homo sapiens platelet at the beginning. This is strong base nature glycoprotein of about 30 molecular weight K which consists of an A chain (molecular weight 16 [about] K) and B chain (molecular weight 14 [about] K), and eight S-S bonds existed [cysteine residue] at a time in both chains at the inside of the peptide of a **** and both chains, and interpeptide, and it has taken stable structure with heat. Main PDGF(s) contained in Homo sapiens platelet granulation are the hetero dimers (PDGF-AB) of the above-mentioned A chain and B chain, this occupies about 70%, a great portion of remainder is the gay dimer (PDGF-BB) of B chain, and the gay dimer (PDGF-AA) of an A chain is also accepted slightly.
[0005] cDNA is already discovered for the above-mentioned A chain and B chain, it is isolated, B chain has the gene product of transformer homing gene v-sis of SSV, and high homology, and this B chain gene is c-sis itself which is the proto oncogene of v-sis.
[0006] On the other hand, the application to the technique which controls gene expression using an antisense oligonucleotide, and its drugs is proposed and considered conventionally.
[0007] The control technique of this gene expression introduces the matter specifically combined with an intracellular target-nucleus acid array, i.e., the antisense matter, in the living body, intercepts the flow of a living body's genetic information, and is regarded as a technique which controls specifically the configuration of protein made into the purpose. If this gene expression control technique is used, the internality or foreignness gene expression made into a target can be controlled specifically, and it is thought that the therapy of the disease produced as a result of this target gene expression or an unsuitable manifestation can be performed. And this technique is capturing the spotlight also from the point that the antisense oligonucleotide used is considered that there is very little especially damage to a living body.
[0008] However, the selection thru/or decision of an antisense array which can do so selection of the antisense oligonucleotide which can control the manifestation of a target cell intentionally, i.e., desired effectiveness, does not have the approach of having been established which predicts this in advance, and it has left the great problem which should be solved in offer of an antisense oligonucleotide. For example, although some attempts in the decision of antisense arrays, such as

the approach [starting area / translation open] of choosing from the untranslation region of the upstream, are proposed, the present condition is a situation's being different with the target gene made into the purpose, and based on selection by trial-and-error after all (reference, such as JP,8-70899,A).
[0009] In addition, the application from control of vascular proliferation to the angiopathy of hypertension etc. is reported and suggested by manifestation control of PDGF by the antisense DNA (the collection of the 16th Japan inflammation society program drafts, Heisei 7, 85 pages). ANSECHINSU DNA concerning this report is antisense oligodeoxynucleotide (ODN) of specific 16 base length of the A chain of PDGF, and desired effectiveness is not done so in a normal-blood-pressure rat.
[0010] As mentioned above, although various antisense techniques are studied and are attracting attention in the gene therapy field and the drugs development field, they do not have the example which is not given to desired manifestation control in PDGF, and was applied to the fibrosis lesion of lungs, such as pneumonitis, of course, either.
[0011]
[Problem(s) to be Solved by the Invention] Therefore, the purpose of this invention is that establishes the use in the antisense oligonucleotide, especially pneumonitis therapy of the request which does the manifestation depressor effect of PDGF so, and it offers.
[0012] In this actual condition, as a result of this invention persons' repeating research wholeheartedly, the antisense oligonucleotide which has the antisense array chosen from the array of the field of the arbitration of the B chain (c-sis) of PDGF completed a header and this invention for having the manifestation depressor effect of PDGF.
[0013]
[Means for Solving the Problem] This invention relates to the platelet derived growth factor manifestation inhibitor which makes an active principle the antisense oligonucleotide to the field of the arbitration of the B chain of a platelet derived growth factor.
[0014] Moreover, this invention relates to the pneumonitis therapy agent containing the antisense oligonucleotide to the field of the arbitration of the B chain of a platelet derived growth factor.
[0015] Hereafter, the display by cable addresses, such as amino acid in this specification, a peptide, a base sequence, and a nucleic acid, shall follow the common use notation in a convention of IUPAC and IUB, "the guideline for creation, such as a specification including a base sequence or an amino acid sequence," (edited by Patent Office), and the field concerned.
[0016]
[Embodiment of the Invention] In this invention, the oligonucleotide which has a complementary array (antisense array) in the array (sense array) of mRNA imprinted from the DNA array of a PDGF-B chain can be included by the antisense oligonucleotide, and this can be an antisense DNA or an antisense RNA. the field where an ANSECHINSU oligonucleotide tends to combine this ANSECHINSU array in this invention as a field of this arbitration to the field of the arbitration of a PDGF-B chain, i.e., the field which cannot take the secondary structure easily (it is easy to take the primary structure), -- be -- it is also good to be able to creep.
[0017] Therefore, as long as hybridization can be effectively carried out in the array of this field (the gene of PDGF and/or the manifestation of protein are controlled in this way), the antisense oligonucleotide of this invention is not limited to the location of that array, die length, qualification-izing, and existence of a mismatch at all.
[0018] The base sequence of this PDGF-B chain is already known (for example, about a Homo sapiens PDGF-B chain). Cell, Vol.39, and 1984, Refer to 89-97;Nature, Vol.316, 22 August 1985, 748-750;Molecular and Cellular Biology, Vol.10, 1990, and 5496-5501 grade, It is desirable to use the target origin and the array corresponding to the origin (seed) according to the mode of use of this invention.
[0019] The ANSECHINSU oligonucleotide used by this invention can be illustrated as an antisense

DNA to the sense array which about 15 to 30 MA (mer) extent including the array of the field of the arbitration of the above-mentioned PDGF-B chain followed.
[0020] Moreover, the ANSECHINSU oligonucleotide used by this invention For example, the phosphorothioate type which replaced one of the oxygen atoms of the phosphoric-acid radical of a phosphodiester bond with the sulfur atom (S-oligo), The methyl FOSUFONETO mold which permuted the oxygen atom of this phosphoric-acid radical by the methyl group (M-oligo), alpha-oligo mold which replaced the phosphate bond with alpha-association, furthermore, it can consider as various gestalten, such as qualification by association of an acridine or the poly lysine, (Biochemistry, 27, 9113-9121(1988);Anti-Cancer Drug Design., and 3 --) 117-127(1988);Nucleic Acids Res., 19, 747-750 (1991), etc.
[0021] As a typical array of an ANSECHINSU oligonucleotide made into an active principle, the antisense array (K) corresponding to the 112nd amino acid sequence can be illustrated, for example from the field of the exon 4 of a PDGF-B chain, i.e., the amino acid sequence field of the 97-180th codons, and a codon 106. This array in Homo sapiens is as being shown in an array table as array number:1, and the array in a mouse is as being shown in an array table as array number:2.
[0022] Moreover, the antisense array (C) over the antisense array (D) over the field of an exon 1, especially the field containing an initiation codon, a field including a splicing site, especially the field between an exon 5 and an exon 6 is mentioned. The array D in Homo sapiens and a mouse is as being shown in array number:3, and the array C in a mouse is as the array C in Homo sapiens being shown in array number:4 array number:5.
[0023] Furthermore, the antisense array (E, H) over the field of exons 1 other than an antisense array (D), the antisense array (A) over the field between an exon 2 and an exon 3, the antisense array (F) over the field of an exon 5, the antisense array (B) over the field between an exon 4 and an exon 5, the antisense array (G) over the field of an exon 6, the antisense array (I) over the field of an exon 7, etc. are mentioned. the array E in a mouse and Homo sapiens -- array number: -- to 6 the array H in a mouse array number:7 the array H in Homo sapiens -- array number: -- to 8 the array A in a mouse array number:9 the array A in Homo sapiens -- array number: -- to 10 the array F in a mouse array number:11 the array F in Homo sapiens -- array number: -- to 12 the array B in a mouse array number:13 the array B in Homo sapiens -- array number: -- it is as the array [in / to array number:16 / in the array / in / to array number:15 / in the array G in a mouse / Homo sapiens / G / a mouse] I showing the array [in / to array number:17 / Homo sapiens] I to 14 array number:18, respectively.
[0024] Array K (field of an exon 4), Array D (field of an exon 1), and Array C (field including a splicing site) are desirable among these arrays.
[0025] In addition, the physical relationship of the array over each number of bases of the antisense oligonucleotide of these typical arrays and which part of a PDGF-B chain gene to be is as being shown in drawing 1 .
[0026] The antisense oligonucleotide made into an active principle in this invention is DNA and/or RNA which consist of this antisense array, and can compound this etc. easily with the solid phase synthesis method using general technique, for example, commercial FOSU follow friend DAITO, commercial hydrogen FOSUFONETO, etc. using an automatic composition machine, etc.
[0027] Moreover, various kinds of above-mentioned qualification-ization can also be performed according to a conventional method, and the commercial reagent for these qualification can also be used suitably.
[0028] Purification of the oligonucleotide obtained in this way can also follow a conventional method, for example, the approach by the usual high performance chromatography, polyacrylamide gel electrophoresis, solvent extraction, a salting-out, etc. can be adopted suitably (J.Am.Chem.Soc., 106, 6077(1984);J.Org.Chem., 50, 390 (1985), etc.). That is, the antisense oligonucleotide used by this invention does not have any limit in the synthesis method or origin, either, as long as desired hybridization can be formed to the sense array of arbitration.

[0029] moreover, various kinds of actuation which may be adopted in manufacture of the antisense oligonucleotide used by this invention, for example, a part, -- each of enzyme processing aiming at the chemosynthesis, this cutting, deletion, addition, or association of a gene, this isolation, purification, duplicates, selections, etc. can follow a conventional method -- [-- for example, a molecular-genetics laboratory procedure and KYORITSU SHUPPAN Co., Ltd. 1983 issue ;P Reference [, such as CR technology and TAKARA SHUZO CO., LTD. 1990 issue,]].
[0030] Thus, the antisense oligonucleotide obtained is useful as the PDGF manifestation inhibitor which is excellent in PDGF manifestation depressant action, and makes this an active principle, especially a pneumonitis therapy agent.
[0031] In addition, the PDGF manifestation depressant action of the antisense oligonucleotide obtained in this way adds the antisense oligonucleotide concerned to the linked transcription translation in which the gene which carries out the code of the PDGF exists, adds the antisense oligonucleotide concerned in the system in which mRNA corresponding to the gene which investigates manifestation inhibition of PDGF or carries out the code of the PDGF exists, and can check it by investigating manifestation inhibition of PDGF.
[0032] Although this can also be used as drugs as it is, generally according to the application as drugs, such as this usual seed DNA reagent, it is usable using the reagent for transgenics, suitable support, etc. by using the above-mentioned active principle as various kinds of constituents.
[0033] It can be used choosing from the excipient of the various kinds permitted pharmacologically usually adopted as these seed drugs as the above-mentioned support on the assumption that it does not have a bad influence on an active principle, an isotonizing agent, a solubilizing agent, a stabilizing agent, antiseptics, an aponia-ized agent, etc. suitably, and especially a gestalt is not limited, either, but it can choose and determine suitably from various kinds of well-known gestalten.
[0034] The active principle of this invention is suitably applied to the various patients who need the treatment by this according to the gestalt. The application can be considered as the direct application to the affected part which requires treatment, and can also be considered as indirect application of the administration in a blood vessel etc. Especially, in the treatment of the fibrosis of lungs, such as pneumonitis, the approach of passing through this invention active principle which is a solution gestalt, for example, and applying directly in respiratory tract can be illustrated preferably.
[0035] Furthermore, the physic of this invention is also usable as a gestalt which used further various kinds of surfactants, such as the enclosure material which meant improvement in durability or membrane permeability, for example, RIPOFE cutin, and liposome, etc. by request.
[0036] The dose of the active principle in this invention can be suitably fluctuated according to the above-mentioned conditions, for example by making about 1-10mg / patient into a standard, although it is suitably set up according to conditions, such as the patient itself and its disease condition, and is not restricted especially.
[0037] In this way, by carrying out administration application of this at a target host's living body, this invention PDGF manifestation inhibitor can aim at desired PDGF manifestation control, and can perform the therapy of the various kinds of diseases and symptoms resulting from a manifestation or its unsuitable manifestation of PDGF. Especially, the PDGF manifestation inhibitor of this invention is suitable for the therapy of pneumonitis, and can control and treat the fibrosis of the lungs in this pneumonitis etc.
[0038] In addition, in this invention, instead of carrying out administration application of the PDGF antisense oligonucleotide as the above-mentioned active principle at a direct living body, this is incorporable into a suitable plasmid vector, and antisense recombinant can be created, or it can include in suitable virus vectors, such as adenovirus and a retrovirus, a desired antisense RNA, i.e., the antisense oligonucleotide of this invention, can also be made to be able to discover within a target host using this etc., and the same curative effect can be done so in this way.
[0039]
[Example] Hereafter, an example and the example of a trial are given and this invention is explained

in more detail.
[0040] Example 1 (preparation of an antisense oligonucleotide)
The array number corresponding to the 106 to 112nd amino acid sequences of a mouse PDGF-B chain (c-sis): The oligonucleotide shown in 2 was compounded with the TETD reagent (applied biotechnology systems company make) as a phosphorothioate type (S-oligo) which permuted one of the oxygen atoms of the phosphoric-acid radical of a phosphodiester bond by the sulfur atom in the automatic DNA synthesizer (380by applied biotechnology systems company A mold).
[0041] S-oligo compounded above carried out isolation purification with reversed phase high pressure liquid chromatography according to the usual approach, and the following trials were presented with it.
[0042] Moreover, the sense oligonucleotide of the above-mentioned array was compounded similarly.
[0043] Since fibrosis happened to lungs when it passed to example of trial 1 mouse and the silica bead was prescribed for the patient in respiratory tract, the effectiveness of the antisense oligonucleotide of this invention was examined by this experiment system as follows.
[0044] The group division of the mouse (C57BL / 6.5 weeks old) was carried out at the following four groups.
[0045] one group: -- a silica -- administration 2 group: -- RIPOFE cutin () [LIFE] Only TECNOLOGIES, Gaithersburg, MD, and the USA Administration 3 group:silica + RIPOFE cutin + antisense oligonucleotide administration 4 group:silica + RIPOFE cutin + sense oligonucleotide administration ****, 16mg/80microl of a silica -- eating raw food -- the micropipette was used, a solution / one animal was prescribed for the patient in pernasality (one group, three groups, and four groups), and the fibrosis of lungs was made to cause experimentally
[0046] 3 hours after the above-mentioned silica administration, antisense oligonucleotide (thing of S-oligo mold prepared in example 1) 7microl (5 mg/mlPBS), 40microl of the sample offering drugs which consist of PBS7microl and RIPOFE cutin 266microl (three groups), And first time administration of the 40microl (four groups) of the control drug agent which consists of sense oligonucleotide 7microl, PBS7microl, and RIPOFE cutin 266microl similarly was carried out into the bronchial tube of a mouse non-invasive under accepting reality, respectively. Moreover, two groups were similarly medicated with RIPOFE cutin independent 40microl instead of the above-mentioned drugs.
[0047] Administration in a bronchial tube of sample offering drugs, a control drug agent, and the RIPOFE cutin independent (the 2nd time) was carried out to two to 4 group respectively similarly after one week from test initiation.
[0048] After two weeks of test initiation, broncho-alveolar lavage (bronchialalveolar lavage:BAL) of the lungs of each group mouse was extracted and carried out, and a hydroxyproline and pathological findings were examined.
[0049] In addition, BAL washed the inside of a bronchial tube with the physiological saline, collected 5ml, and examined the total cell number and a cell fraction. Moreover, it bred to the experiment mouse and it was made to carry out free baiting by SPF (aseptic condition) during an experiment period.
[0050] The result was as follows.
[0051] (1) The amount of hydroxyprolines under lung tissue : the bar graph which calculated the amount of hydroxyprolines of the lungs of each group mouse (mug) is shown in drawing 2 .
[0052] In the comparison of the amount of hydroxyprolines measured from drawing 2 in order to evaluate the fibrosis of lungs objective By the silica independent administration group (one group), 819.5**124.6(an average of**SD) mug / lungs, By the RIPOFE cutin independent administration group (two groups), 281.7**31.7microg / lungs, By the silica + RIPOFE cutin + antisense oligonucleotide administration group (three groups), 498.2**41.3microg / lungs, It became 513.2**42.1microg / lungs by the silica + RIPOFE cutin + sense oligonucleotide administration group

(four groups), and three groups and four groups were falling intentionally compared with one group. Moreover, the measured value of two groups was equivalent to the measured value of the normal mouse which has not received administration of a silica.
[0053] (2) Analysis of the cell component in BAL liquid : according to the analysis of this cell component, compared with one group, it has stopped at three groups accepting the fall inclination of neutrophil leucocyte to be the significant fall of a total cell number.
[0054] (3) Pathological findings : a result is shown in drawing 3 (one group), drawing 4 R> 4 (three groups), and drawing 5 (four groups).
[0055] From these drawings, infiltration of the prominent inflammatory cell by the silica bead administration currently seen by one group disappeared mostly by three groups, and was slight-illness-ized by four groups.
[0056] (4) Consideration : disappearance of the lesion in a significant fall and pathological findings of the amount of hydroxyprolines under lung tissue which is the index of the fibrosis of lungs by passing through an antisense oligonucleotide with RIPOFE cutin, and prescribing it for the patient from the above result in respiratory tract to the pneumonitis and the lung fibrosis model caused by the silica of a mouse was accepted.
[0057] An antisense oligonucleotide acts effectively and this fact is considered to have controlled nearly completely the inflammation and fibrosis of lungs which are guided by the silica through production control of PDGF.
[0058] That is, in the result of this experiment, the antisense oligonucleotide of PDGF suggests strongly that it is effective as a remedy of pneumonitis and lung fibrosis.
[0059] The oligonucleotide (A-K) shown in the example 2 array numbers 2, 3, 4, 6, 7, 9, 11, 13, 15, and 17 was compounded like the example 1.
[0060] example of trial 2 configuration ---like -- PDGF-B Mouse which has discovered mRNA monocyte-macrophagecell line it is -- the various PDGF-B antisense oligonucleotides which compounded this in the example 2 by the following approaches, using J774 cell as an ingredient -- 6 and 14 or 22 hours -- processing -- PDGF-B of J774 cell mRNA level was investigated by the slot blot hgbridization.
[0061] (1) Preparation SolutionA:500microM of PDGF antisense oligonucleotide-RIPOFE cutin reagent complex The PDGF antisense oligonucleotide was diluted with RPMI-1640 (antibiotic non-**) which contains BSA 0.02% 50 times, and 20microl preparation of 10microM was done (a part for 1well).
[0062] SolutionB: 4micro (LIFE TECHNOLOGIES) of RIPOFE cutin reagents I was diluted with RPMI-1640 (antibiotic non-**) which contains BSA 0.02% 5 times (a part for 1well), and it was left at the room temperature for 45 minutes.
[0063] Churning mixing of SolutionA and the SolutionB was carried out quietly, and it incubated at the room temperature for 15 minutes. 160microl addition of RPMI-1640 (antibiotic non-**) which contains BSA in this mixed solution 0.02% was done after 15 minutes (the total capacity: 200microl).
[0064] J774 cell -- 105cells(es)/-- it wound 0.18 ml/well at a time around 96well plate, and cultivated by 37 degrees C and 5% CO2 incubator. 24 hours after, centrifugal was carried out at 20 degrees C, and the culture supernatant was washed twice by 200microl/well for 500rpm and 5 minutes using RPMI-1640 (antibiotic non-**), after carrying out suction removal. above-mentioned PDGF-B antisense oligonucleotide-RIPOFE cutin reagent complex -- J774 cell -- every [200microl/well] -- it added. Every 24 wells per one kind of PDGF-B antisense oligonucleotide-RIPOFE cutin reagent complex were used. 37 degrees C and 5% CO2 incubator -- after 6 and 14 or 22-hour culture and a culture supernatant -- throwing away -- ISOGEN (NIPPON GENE) -- every [50microl/well] -- it added and the RNA solution was prepared according to the protocol (therefore, about one kind of PDGF antisense oligonucleotide, J774 cell is 2.4x106cells and 1.2ml of ISOGEN(s) was used). The outline of the preparation of an RNA solution is carried out to below.
[0065] Chloroform is added into the cell which carried out ISOGEN processing. Violently After

churning, Carry out centrifugal separation (12,000rpm, 10min., 4 degrees C), move the upper layer to a new tube, and isopropanol is added. To 12,000rpm, 10min., and the precipitate that carried out centrifugal separation and that was obtained at 4 degrees C, ethanol was added 80%, after Vortex, centrifugal was carried out at 7,500rpm, 6min., and 4 degrees C, supernatant liquid was thrown away, precipitate was air-dried, distilled water was added, and the RNA solution was prepared.
[0066] (2) To 8micro [of slot blot-hgbridization RNA solutions] g/16microl, formamide 32microl, formaldehyde 11.2microl, and 20xSSC(after dissolving NaCl 175.32g and 88.23g of sodium citrates in distilled water and being referred to as 1l., autoclave sterilization is carried out and it prepares) 3.2microl were added, and 68 degrees C incubated after churning to it for 15 minutes. 124.8microl After 15 minutes, it ice-cooled for 3 minutes and 20xSSC was added.
[0067] TRANSFER beforehand dipped in 20xSSC the object for PDGF-B after washing MEMBRANE (PALLBIOSUPORT, DIV.) by 10xSSC of 500microl/slot -- every [140microl/slot] and the object for beta-Actin -- every [40microl/slot] -- it added.
[0068] It washed twice by 10xSSC of 300microl/slot after adding the sample solution, respectively. TRANSFER which Blot(ed) MEMBRANE is PDGF-B which carried out the label after pre hybridization at 68 degrees C after UV irradiation 32P using ExpressHybsolution (CLONTECH Laboratories, Inc.). DNAprobe or beta-Actin DNA Hybridization was carried out by probe. After hybridization termination, TRANSFER 0.1xSSC and 0.1%SDS washed 50 degree C of MEMBRANE(s) for 40 minutes after washing for 40 minutes by 2xSSC and 0.1%SDS. Washed TRANSFER MEMBRANE is GS-525. Sample Loading After fixing to Exposurepad in Dock and Expos(ing) to Screen, using GS-525MolecularImagerSystem, PDGF-BmRNA level was measured and it amended to beta-Actin mRNA level.
[0069] (3) Result : the result of a slot blot hgbridization was shown in drawing 6 . The axis of ordinate shows % of the ratio of PDGF-B/beta-Actin in J774 cell which processed the ratio of PDGF-B/beta-Actin in J774 cell which added distilled water instead of a PDGF-B antisense oligonucleotide and RIPOFE cutin by each PDGF-B antisense oligonucleotide when considering as 100%. When it incubated from this drawing for 14 hours, it turned out that there are many PDGF-B antisense oligonucleotides which show strong control.
[0070] When the result of 6 and 14 or 22-hour processing is compared synthetically, it is a PDGF-B antisense oligonucleotide. - C and D are PDGF-B from K. It turned out that strong control of mRNA level is shown. Moreover, PDGF-B antisense oligonucleotide [- The operation of C and D was judged not to be what is depended on the toxicity of an oligonucleotide.] - It is a mismatch to C, D, and coincidence. - C, D, and sense - From the result of the slot blot hgbridization using C and D to a PDGF-B antisense oligonucleotide Therefore, PDGF-B antisense oligonucleotide It is under examination about the effectiveness that it can set. - C and D are chosen and it is in vivo. A PDGF-B antisense oligonucleotide, a mismatch, and sense - The array of C and D was shown in drawing 7 .
[0071]
[Layout Table]
[0072] array number: -- die-length [of one array]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: GTCTATGAGG CGCCGGGAGA T 21 [0073] array number: -- die-length [of two arrays]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: ATCGATGAGG TTCCGCGAGA T 21 [0074] array number: -- die-length [of three arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: AGCAGCGATT CATGCCGACT CC 22 [0075] array number: -- die-length [of four arrays]: -- mold [of 25 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: TGGGAAGGCA GCTTACCTCG CTGCT 25 [0076] array number: -- die-length [of five arrays]: -- mold [of 25 arrays]: -- number [of nucleic-acid chains]: -- single

strand topology: -- class [of straight-line-like array]: -- DNA Array: CTAGAAAGGT GGTTACCTCG CTGCT 25 [0077] array number: -- die-length [of six arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: AGAGCGCCCA GCAGCGATTC AT 22 [0078] array number: -- die-length [of seven arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: TCGGGTCAGT CTGTCTATCT AC 22 [0079] array number: -- die-length [of eight arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: GCGAGTCCGT CGGTCCGTCT GC 22 [0080] array number: -- die-length [of nine arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: GGTCCGATTT ACCTACGGAG TC 22 [0081] array number: -- die-length [of ten arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: GATTCCATTT ACCTCCGGGG TC 22 [0082] array number: -- die-length [of 11 arrays]: -- mold [of 25 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: GAGGTGGTCC TCCAAGGTCA CTGTG 25 [0083] array number: -- die-length [of 12 arrays]: -- mold [of 25 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: CAGGTGGTCT TCCAGCGTCA CCGTG 25 [0084] array number: -- die-length [of 13 arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: ATCTTTCTCA CCTGGAGGAC AA 22 [0085] array number: -- die-length [of 14 arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: ATCTTTCTCA CCTGGAGGAC AG 22 [0086] array number: -- die-length [of 15 arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: CGCCTTGTCA TGGGTGTGCT TA 22 [0087] array number: -- die-length [of 16 arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: CGTCTTGTCA TGCGTGTGCT TG 22 [0088] array number: -- die-length [of 17 arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: CAACATTATC ACTCCAAGGA CC 22 [0089] array number: -- die-length [of 18 arrays]: -- mold [of 22 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight-line-like array]: -- DNA Array: CAATATTATC TACTCCAAGG CC 22

[Translation done.]

* NOTICES *

JPO and NCIPI are not responsible for any damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] The typical array of the antisense oligonucleotide used by this invention is drawing showing the physical relationship of the array over which part of a PDGF-B chain gene to be. The part which attached the number in drawing shows an exon.
[Drawing 2] It is the graph which shows the amount of hydroxyprolines under lung tissue of each group mouse called for according to the example 1 of a trial.
[Drawing 3] In the example 1 of a trial, it is the photograph in which the ecology of a living thing which observed the extraphysiologic view of the lung tissue of one group is shown.
[Drawing 4] In the example 1 of a trial, it is the photograph in which the ecology of a living thing which observed the extraphysiologic view of the lung tissue of three groups is shown.
[Drawing 5] In the example 1 of a trial, it is the photograph in which the ecology of a living thing which observed the extraphysiologic view of the lung tissue of four groups is shown.
[Drawing 6] It is the graph which shows the result of the slot blot hgbridization in the example 2 of a trial.
[Drawing 7] It is drawing showing the array of D in a PDGF-B antisense oligonucleotide, a mismatch, and a sense C list.

[Translation done.]

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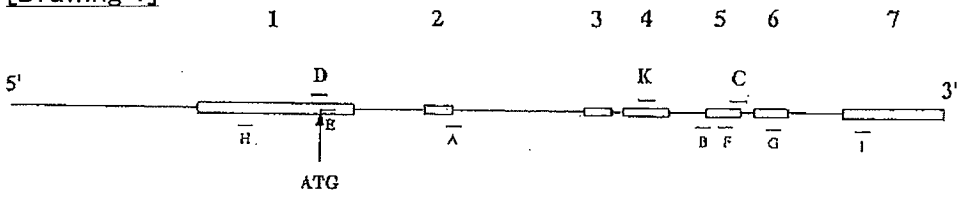
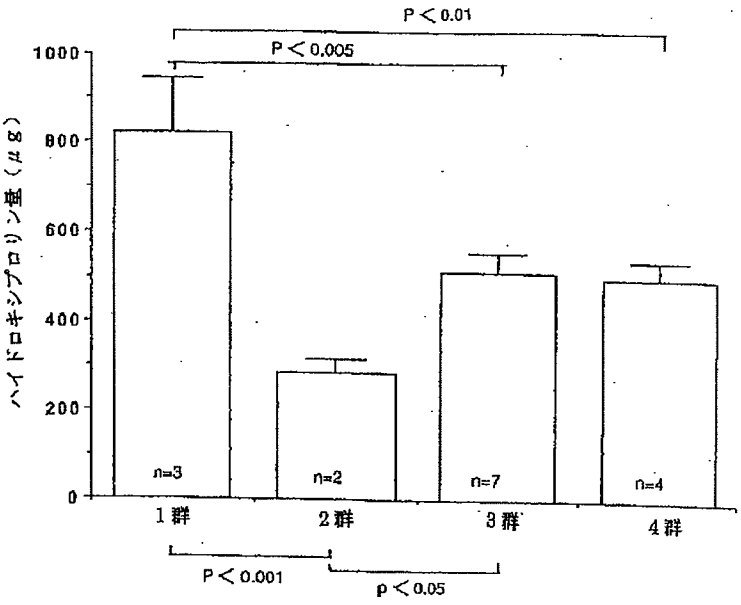
DRAWINGS

[Drawing 1]

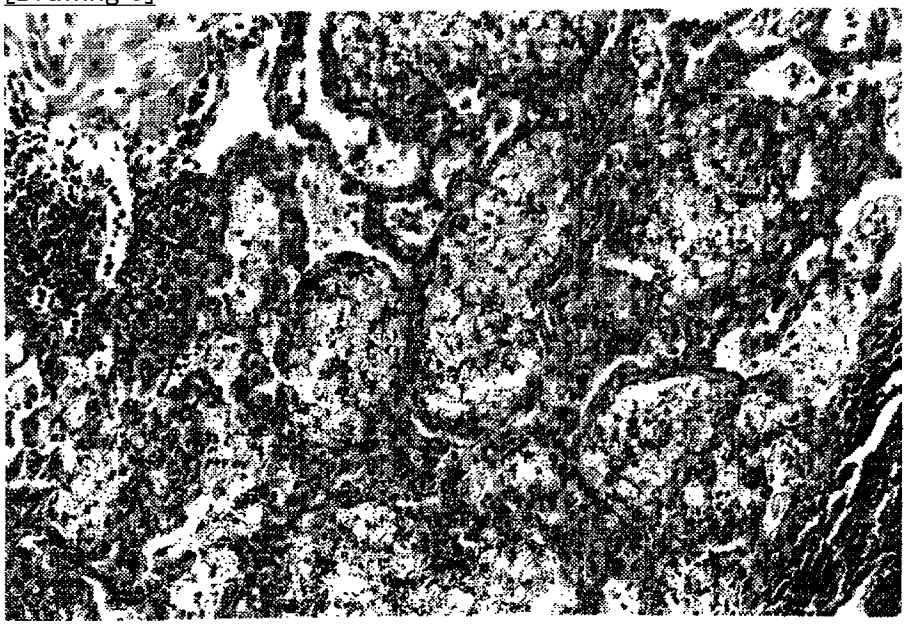


アンチセンスオリゴヌクレオチド	塩基数
A	22
B	22
C	25
D	22
E	22
F	25
G	22
H	22
I	22
K	21

[Drawing 2]

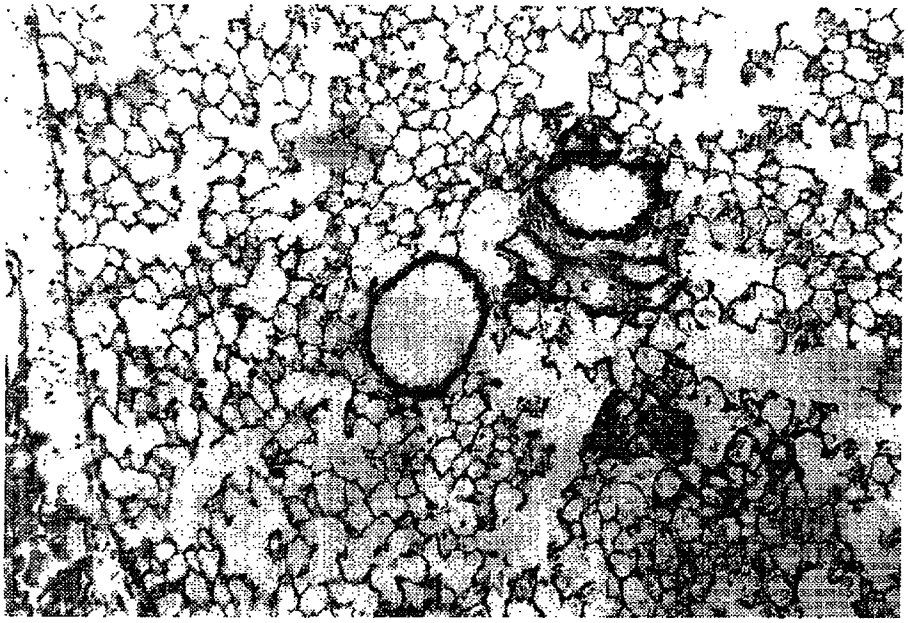


[Drawing 3]



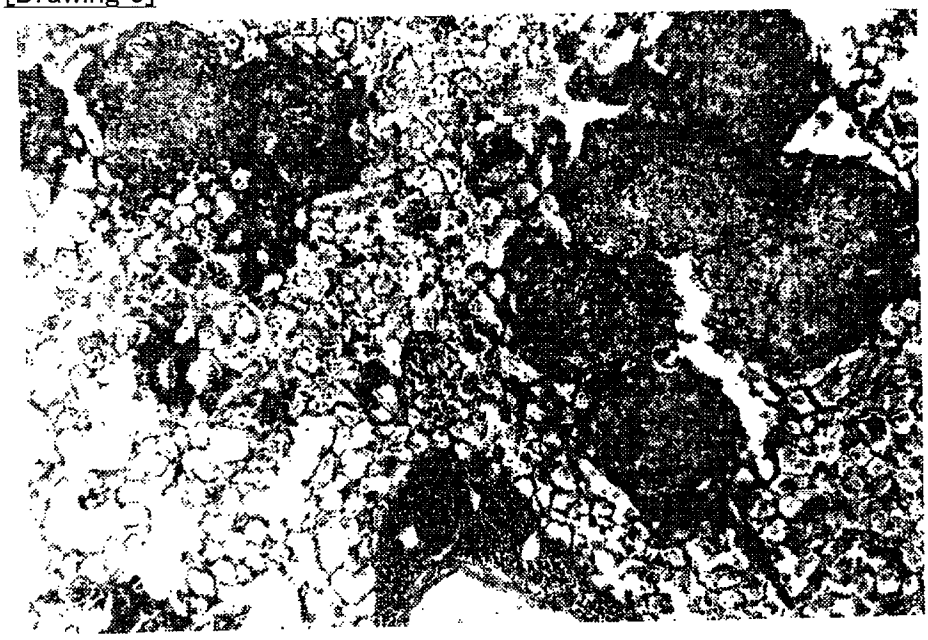
図面代用写真

[Drawing 4]



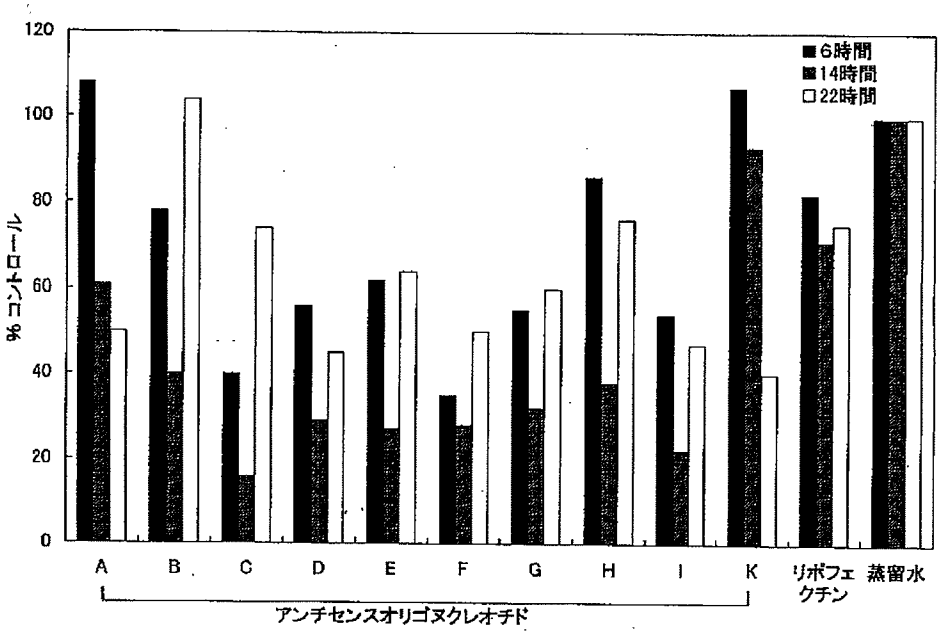
図面代用写真

[Drawing 5]



図面代用写真

[Drawing 6]



[Drawing 7]

[マウスPDGF－B鎖のスプライシングサイト]

C－センマ : 5'-AGC AGC GAG GTA AGC TGC CTT CCC A-3' (25-mer)
(5442, 5466)

C－アンチセンマ: 5'-TGG GAA GGC AGC TTA CCT CGC TGC T-3' (25-mer)

C－ミスマッチ : 5'-TCG GAT GGC AGA TGA CTT CGC CGC T-3' (25-mer, 6bp)

[マウスPDGF－B鎖のコード領域]

D－センマ : 5'-GGA GTC GGC ATG AAT CGC TGC T-3' (22-mer)
(3037, 3058)

D－アンチセンマ: 5'-AGC AGC GAT TCA TGC CGA CTC C-3' (22-mer)

D－ミスマッチ : 5'-CGC ATC GAT TCA AGC TGA CGC C-3' (22-mer, 5bp)

[Translation done.]